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CHEMICAL CONSTITUENTS FROM FRUITS OF *HYDNOCARPUS HAINANENSIS* MERR. (FLACOURTIACEAE) IN VIETNAM

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Abstract

Five compounds were isolated from the fruits of *Hydnocarpus hainanensis* Merr. Sleum. (Flacourtiaceae). Their structures were determined by spectroscopic analysis including MS and NMR. The isolates were identified as taraktophyllin (**1**), hydnocarpic acid (**2**), 3,4-dihydroxybenzyl alcohol (**3**), 3,4-dihydroxybenzoic acid (**4**) and 3-hydroxy-4-methoxybenzoic acid (**5**).

Keywords. *Hydnocarpus hainanensis*, Flacourtiaceae, cyclopentenoid cyanohydrin glucosides.

1. INTRODUCTION

Flacourtiaceae comprises about 89 genera with 1300 species found throughout the tropical and temperate regions of the world [1]. Genus of *Hydnocarpus* consists of about 40 species, many of them have been used in folk medicine [2]. Previous studies showed that the genus *Hydnocarpus* contains flavonoligans, flavonones, phenolic and acid chaumoorgic which exhibited antibacterial, antioxidant and anticancer activities [3-7]. In continuation of our research of bioactive compounds from the plants of Flacourtiaceae family, further purification of the crude extract of *Hydnocarpus hainanensis* fruits has led to the isolation of five compounds **1-5**.

2. MATERIAL AND METHODS

2.1. General experimental procedures

Optical rotations were recorded on a Polax-2 L polarimeter. Melting points were determined using a Buchi B-545 instrument. ESI-MS were obtained on an Agilent 1100 LC-MSD Trap spectrometer. The NMR spectra were recorded on Bruker 500.13 MHz spectrometer, operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C NMR, respectively.

2.2. Plant material

Fruits of *H. hainanensis* Merr. were collected from Quang Tri, Vietnam in November 2006. A voucher specimen (VN-1761) was deposited at the Institute of Ecology and Biological resources, Vietnam Academy Science and Technology.

2.3. Extraction and isolation

Dry powders (0.65 kg) of the fruits of *H. hainanensis* were extracted with ethanol (5 × 1.5 L). The solvents were removed under diminished pressure. The residue (101.7 g) was suspended in water (0.5 L) and then partitioned successively with *n*-hexane, EtOAc. The *n*-hexane, EtOAc and water solutions were concentrated to dryness, affording 43 g, 20 g and 30 g, respectively.

n-Hexane extract (43 g) was fractionated by column chromatography (CC) on silica gel, eluting with *n*-hexane/EtOAc gradient to yield 8 fractions. Fractions 3 (1.7 g) was purified on silica gel CC, eluted with CH_2Cl_2/EtOAc gradient to obtain compound **5** (23 mg).

EtOAc extract (20 g) was subjected to CC on silica gel, eluted with CH_2Cl_2/MeOH gradient to

furnish 7 fractions. Fraction 3 (1.1 g) was purified by CC on silica gel (CH_2Cl_2/acetone gradient) to afford compound **7** (5 mg). Fraction 4 (1.3 g) was separated on silica gel CC (CH_2Cl_2/MeOH gradient), followed by preparative TLC (CH_2Cl_2/MeOH: 95/5) to obtain compound **8** (7 mg). Fraction 5 (1.6 g) was purified on silica gel CC (CH_2Cl_2/MeOH gradient), following by CC on Sephadex LH-20 (MeOH) affording compound **6** (7 mg).

Water extract (30 g) was chromatographed on C-18 (MeOH/H_2O gradient) to give 6 fractions. Fraction 4 (1.1 g) was separated by CC on Sephadex to afford two subfractions. Subfraction 1 (0.6 g) was separated by CC on Sephadex LH-20 following by preparative TLC (CH_2Cl_2/dioxane: 9/1) to give compound **2** (7 mg) and **3** (8 mg). Subfraction 2 (0.3 g) was purified by CC on Sephadex LH-20 (MeOH) to obtain compound **1** (5 mg). Fraction 5 (1.8 g) was separated by Sephadex LH-20 CC (MeOH) yielding two subfractions. Subfraction 2 (0.6 g) was subjected to CC on silica gel (CH_2Cl_2/MeOH gradient), followed by Sephadex LH-20 CC (MeOH) to give compound **4** (5.5 mg). Fraction 3 (350 mg) was separated by CC on silica gel, eluted with *n*-hexane/acetone gradient to give compound **6** (59 mg).

Taraktophyllin (1): Colorless syrup; $[\alpha]_D$ -181 (c 0.083, MeOH). ESI-MS (*m/z*): 310.0 $[M+Na]^+$. ^{1}H-NMR (500 MHz, CD_3OD): 6.16 (1H, dd, *J* = 5.5 and 1.5 Hz, H-2), 6.25 (1H, dd, *J* = 5.5 and 2.0 Hz, H-3), 4.83 (1H, m, H-4), 2.25 (1H, dd, *J* = 4.5 and 14.5 Hz, H-5), 3.06 (1H, dd, *J* = 6.0 and 14.5 Hz, H-5), 4.69 (1H, d, *J* = 8.0 Hz, H-1'), 3.24 (1H, dd, *J* = 7.5 and 9.0 Hz, H-2'), 3.40 (1H, s, H-3'), 3.36 (1H, s, H-4'), 3.36 (1H, s, H-5'), 3.69 (1H, dd, *J* = 5.0 and 11.5 Hz, H-6'a), 3.89 (1H, dd, *J* = 1.5 and 11.5 Hz, H-6'b). ^{13}C-NMR (125 MHz, CD_3OD): 82.5 (C-1), 132.9 (C-2), 142.3 (C-3), 74.1 (C-4), 47.9 (C-5), 101.4 (C-1'), 74.7 (C-2'), 77.9 (C-3'), 71.3 (C-4'), 78.2 (C-5'), 62.6 (C-6'), 120.1 (CN).

Hydnocarpic acid (2): Fatty oil $[\alpha]_D$ +36 (c 0.20, CH_2Cl_2). ESI-MS (*m/z*): 253.0 $[M+H]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.68 (m, CH-1,2), 2.60 (m, CH-5), 2.34 (t, CH_2-15), 2.30 (m, H-3a), 2.22 (m, H-3b), 2.00 (m, H-4a), 1.38 (m, H-4b), 1.62 (quint, *J* = 7.5 Hz, CH_2-14), 1.26 (8x CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): 179.2 (C-16), 135.5 (C-1), 130.0 (C-2), 45.6 (C-5), 36.2 (C-6), 33.9 (C-15), 32.0 (C-3), 29.9 (C-4), 29.6-28.0 (C7-C13), 24.2 (C-14).

3,4-Dihydroxybenzyl alcohol (**3):** Colorless oil. ESI-MS (*m/z*): 140 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD): 8.02 (d, *J* = 2.5 Hz, H-2), 7.37 (d, *J* = 8.5 Hz, H-6), 7.24 (d, *J* = 2.5, 8.5 Hz, H-5), 4.60 (CH_2-O).

3,4-Dihydroxybenzoic acid (**4):** White powder. ^{1}H-NMR (500 MHz, CD_3OD): 7.45 (d, *J* = 1.5 Hz, H-2), 7.43 (dd, *J* = 1.5 Hz and 8.0, H-6), 6.80 (d, *J* = 8.0 Hz, H-5).

3-Hydroxy-4-methoxybenzoic acid (5): Light powder. ESI-MS (m/z): 169.1 $[M+H]^+$. ^{1}H- NMR (500 MHz, CD_3OD): 7.58 (1H, d, *J* = 1.7 Hz, H-2), 7.55 (1H, dd, *J* = 8.2 and 1.7 Hz, H-6), 6.84 (dd, *J* = 8.2 Hz, H-5), 3.91 (3H, s, OCH_3).

3. RESULTS AND DISCUSION

Compound **1** was optically active, $[\alpha]^{30}_D$ -181 (c 0.083, MeOH). Its ESI-MS (positive) indicated the pseudo-molecular ion peak at *m/z* 310.0 $[M+Na]^+$. The ^{1}H-NMR spectrum of **1** showed the signals of oxymethine protons from 3.24 to 4.69 ppm, two olefinic protons at δ_H 6.16 (dd, *J* = 5.5 and 1.5 Hz, H-2) and 6.25 (1H, dd, *J* = 5.5 and 2.0 Hz, H-3), and two protons of a methylene at δ_H 2.25 (dd, *J* = 4.5 and 14.5 Hz, H_a-5) and 3.06 (dd, *J* = 6.0 and 14.5 Hz, H_b-5). Analyses of the ^{13}C-NMR and DEPT spectra with the aid of the HSQC spectrum of **1** indicated the presence of 12 carbons, including six oxymethines (one annomeric methine at δ_C 101.4, C-1'), two methylene groups (one of them was linked to oxygen as indicated by its chemical shifts (δ_C 62.6 and δ_H 3.69, 3.89, CH_2-6'), an oxygenated quaternary carbon (δ_C 82.5, C-1), a double bond and a nitrile functionality (δ_C 120.1, C-7). This observation suggested the presence of a sugar moiety. Analysis of the COSY spectrum of **1** revealed two spin-spin coupling systems: correlations of a sugar moiety and a connection starting from H-2 (δ_H 6.16) to CH_2-5 (δ_H 2.25, 3.06). Analysis of the coupling constants and chemical shifts of the sugar moiety [4.69 (d, *J* = 8.0 Hz, H-1'), 3.24 (dd, *J* = 8.0, 7.5 Hz, H-2'), 3.40 (t, *J* = 7.5 Hz, H-3'), 3.33 (overlapped, H-4' and H-5'), 3.69 (dd, *J* = 5.5, 12.0 Hz, H_a-6'), 3.89 (dd, *J* = 2.0, 12.0 Hz, H_b-6')] determined the presence of glucopyranose moiety in the structure of **1**. In the HMBC spectrum of **1**, the correlations of the protons H-2 (δ_H 6.16) and CH_2-5 (δ_H 2.25, 3.06) with C-1 (δ_C 82.5) and the nitrile carbon C-7 (δ_C 120.1) indicated the formation of the cycloheptene ring. The glucopyranosyl moiety was bonded to C-1 as shown by cross-peak of H-1'' (δ_H 4.69) with C-1 in the HMBC spectrum. The β-configuration of glucopyranosyl moiety was established by anti coupling constant of H-1'' (*J* = 8.0 Hz). Detailed analyses of the 2D NMR spectra and comparison of the NMR data and optical

rotation with reported values indicated the structure of **1** as taraktophyllin which was previously described [8].

Compound **2** was isolated as colorless oil and optically active, $[\alpha]_D$ +36 (*c* 0.20, CH_2Cl_2). Its ESI-MS indicated the pseudo-molecular ion at *m/z* 253 $[M+H]^+$. In the ^1H NMR spectrum, the signals of two olefinic protons at δ_H 5.68 (H-1 and H-2) and the complex overlapped signals of protons in the aliphatic region were observed. The ^{13}C and DEPT of **2** indicated the presence of a carboxylic carbon at δ_C 179.2 (C-16), two olefinic carbons at δ_C 135.5 (C-1), 130.0 (C-2), a sp^3 methine at δ_C 45.6 (C-5), and twelve sp^3 methylenes. Analysis of COSY spectrum of **2** defined the presence of a cyclopentene ring by a connection from H-2 (δ_H 5.68) to H-5 (δ_H 2.60) via H-2, CH_2-3 and CH_2-4. Thus, the remaining signals were assigned to the undecanoic acid side chain. The linkage of C-5/C-6 was determined by correlation of C-6 (δ_C 36.2) with H-1 in the HMBC spectrum. Complete analyses of the 2D NMR spectra allowed establishing the structure of **2** as hydnocarpic acid. This compound was previously isolated from several species of *Hydnocarpus* genus [9].

Compound **3** was obtained as colorless oil. The ^1H NMR of **3** displayed the signals of an ABX aromatic system [δ_H 8.02 (d, *J* = 2.5 Hz, H-2), 7.37 (d, *J* = 8.5 Hz, H-6), 7.24 (d, *J* = 2.5, 8.5 Hz, H-5)] and two protons of an oxymethylene as singlet at δ_H 4.60. These NMR data were in agreement with those of 3,4-dihydroxybenzyl alcohol [10].

The ^1H NMR spectrum of **4** also exhibited the presence of an ABX aromatic system as **3**, forming from three protons at δ_H 7.45 (d, *J* = 1.5 Hz, H-2), 7.43 (dd, *J* = 1.5 Hz and 8.0, H-6) and 6.80 (d, *J* = 8.0 Hz, H-5). However, the signal of the oxymethylene was not observed in the ^1H NMR spectrum of **4**. This strongly suggested that the oxymethylene was oxidized into carboxylic acid group. This suggestion was confirmed by comparison of NMR data of **4** with those of previously reported for 3,4-dihydroxybenzoic acid [11, 12].

^1H-NMR spectrum of **5** indicated the signals close to those of **4**, except for the presence of an additional methoxy signal at δ_H 3.91. The ABX system was formed by signals of H-2 (δ_H 7.58, d, *J* = 1.7 Hz), H-5 (δ_H 7.55, dd, *J* = 8.2 and 1.7 Hz) and H-6 (δ_H 6.84, dd, *J* = 8.2 Hz). Comparison of the NMR data with reported data in the literature indicated the structure of **5** as 3-hydroxy-4-methoxybenzoic acid [12].

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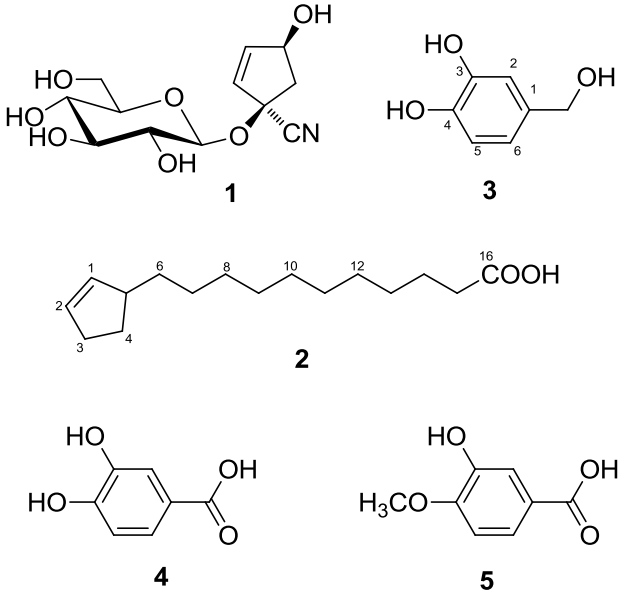

Figure 1: Structures of compounds **1-5** isolated from the fruits of *Hydnocarpus hainanensis* Merr.

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